

UNIVERSITE DE GENEVE
Département de médecine

FACULTE DE MEDECINE
Professeur A.-F. MULLER

In vitro stimulation and inhibition of renin release by human kidney slices

THESE

présentée à la Faculté de médecine de l'Université de Genève
pour obtenir le grade de docteur en médecine

par

Eric ROSSET

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THONEX (Suisse)

Thèse N° 3385

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La Faculté de médecine, sur le préavis des professeurs R. Veyrat et Alex F. Muller, autorise l'impression de la présente thèse, sans prétendre par là émettre d'opinion sur les propositions qui y sont énoncées.

Genève, le 28 janvier 1974

Le doyen :
William Geisendorf

Thèse No 3385



Nous remercions vivement le professeur A.-F. Muller (Directeur du Département de médecine interne, Genève) pour l'honneur qu'il nous a fait en présidant cette thèse, et pour l'aide qu'il nous a apportée durant la phase finale, en tant que chef ad interim de la Division de néphrologie et d'hypertension, après le décès du professeur R. Veyrat.

Nous exprimons notre vive reconnaissance au professeur R. Veyrat, tragiquement décédé durant le printemps 1973 alors que la rédaction de cette thèse était pratiquement terminée. Nous en avons eu ensemble l'idée lors du IVème Congrès International de Néphrologie de Stockholm en juin 1969. Durant les deux années qui suivirent le professeur R. Veyrat en a dirigé l'élaboration avec une grande compétence et en ne cessant de nous témoigner une bienveillante amitié. Tant comme étudiant, que comme assistant au Laboratoire de physiopathologie, nous avons fait notre chemin durant cinq années aux côtés du professeur R. Veyrat, qui par sa rigueur scientifique, sa droiture et la générosité qu'il a marqué à notre égard et à celui de notre femme, restera longtemps pour nous un exemple.

AU PROFESSEUR ROBERT VEYRAT

INTRODUCTION

The physiological mechanisms regulating renin production and release by the kidney are multiple. Three main theories have been proposed for the control of renin release (RR). I. the baroreceptor theory (2). II. the macula densa theory (3, 4). III. the sympathetic nervous system theory (5, 6). In most clinical and experimental conditions blood volume (baroreceptor theory) seems to be the dominant feature among the various factors regulating RR. But modulation of renin secretion may still depend on other important mechanisms. Several drugs have been studied in respect to their effect on RR. In vivo two vasoactive hormones, norepinephrine (NE) and angiotensin II (AII) have opposite effects on RR; NE stimulates (5) and AII inhibits RR (7, 8). Administration of ADH has been reported to be in vivo a powerful inhibitor of renin release (9, 10).

In vivo effect on RR of d-aldosterone, a mineralo-corticoid hormone, depends probably on sodium intake; in conjunction with a salted diet it decreases RR, but it has no evident effect with strictly salt free diet (11, 12).

Theoretically, the above hormones can act in vivo by a direct mechanism on the renin producing cells, or by an indirect mechanism, for example: hemodynamic changes, sodium concentration or osmolarity variations at the level of the macula densa. If some of these hormones act by a direct mechanism on juxtaglomerular apparatus, it should be possible to demonstrate this effect in an in vitro model of kidney slices. We have studied the effect of these hormones on RR in an in vitro system, utilizing human kidney cortex slices incubated in a Krebs-Ringer bicarbonate medium (KR). Successive incubations of the same slice in media of varying compositions allowed a "kinetic" analysis of RR into the incubation medium.

In vitro, NE, NE plus theophylline (Th) and dibutyryl cyclic AMP provoked a marked increase in RR, AII a marked inhibition, whereas aldosterone, ADH and angiotensin I (AI) had no effect.

MATERIALS AND METHODS

1. Incubation of kidney slices

a) Tissue samples : twelve different human kidneys obtained by surgical nephrectomy were used. Nephrectomies were performed because of renal arterial thrombosis (cases No 1, 3 and 9), hydronephrosis with malignant hypertension (case No 2), pyelonephritis with lithiasis (case No 5), renal tuberculosis (cases No 4, 6 and 7), chronic glomerulonephritis with malignant hypertension on chronic hemodialysis (case No 8), renal laceration (case No 10), chronic pyelonephritis with intractable hypertension on hemodialysis (case No 11) and intractable malignant hypertension (case No 12). Case No 6 (renal tuberculosis) gave no renin activity in the incubation media.

b) Preparation of kidney slices : Immediately after nephrectomy the kidneys were kept cool in saline (4°C), and at the latest two hours after, cortex was cut into slices and conserved in the same medium. Width of the slices varied

from 0.2 mm to 0.7 mm, cut manually with a razor blade. Their wet weight at the beginning varied from 100 mg to 400 mg. Incubation of the slices started between one to three hours after nephrectomy.

c) Incubation of kidney slices (experimental design) : A single kidney slice was incubated into KR in a non-siliconated Erlenmeyer with a gas phase on the surface of oxygen 95 % and CO₂ 5 %. The preparation was smoothly shaken in a bain-marie at a constant temperature of 37° C. The experimental protocol began with a 15 minutes preincubation period, then each slice was incubated for three to four successive 30 minutes periods (incubation I, II, III and IV) in 14 ml of KR and 1 ml of human serum from a pool of serum. At the end of each period the incubation medium was collected and the flask washed with 1 ml of fresh solution. The rinsing liquid was added to the previously collected incubation medium and immediately frozen at -20° C and conserved at this temperature until assay for renin activity. The renin activity measured in these solutions corresponds to the RR during 30 min of incubation.

Each slice was successively incubated during four periods of 30 min. with fresh solution for every period. The hormones to be studied were added during incubation period II. Incubation period I represented basal RR, and incubation periods III and IV the restitution periods of basal RR after removal of the added hormones.

d) Krebs-Ringer Bicarbonate medium : Incubation medium was Krebs-Ringer buffer bicarbonate medium, freshly prepared from the following stock solution : 100 parts of 0.9 % NaCl, 2.5 parts of solution 1.15 % KCl, 3 parts of solution of 1.22 % CaCl₂, one part of solution of 2.11 % KH₂PO₄, one part of solution of 3.82 % MgSO₄ 7H₂O, 21 parts of solution of 1.3 % NaHCO₃ gased with CO₂ for one hour, freshly prepared for each experiment; the solution was then gased with 5 % CO₂ and 95 % O₂ and kept at 37° C until its use. After adding 200 mg glucose for 100 ml of solution the pH was adjusted at 7.4-7.3, Na, K and Ca concentrations were also measured (Na range : 135-150 mEq/l, K : 4-5 mEq/l, Ca 75-90 mg/l).

e) Pool of human serum : A pool of serum of 250 ml has been constituted with blood from three normal subjects, regular blood donors at the Centre de Transfusion Sanguine of Geneva, on unrestricted diet, without hypertension. Fibrin was taken away by coagulation and filtration on glass wool. The pool was stored at -20° C.

Adding 1 ml of serum to KR has proved to be useful in stabilizing the renin liberated during the incubation.

f) Hormone solutions : Solutions of hormones prepared in KR solution were added at three intervals (at 0, 10, and 20 min of incubation period II). Concentration in incubation flask were : 1) norepinephrine (Arterenol® Hoechst) : 4.10^{-6} M; 2) angiotensin II (Hypertensin® Ciba) : $0.7.10^{-6}$ M; 3) isoleucine-5 angiotensin I (Lot 7004, Schwarz Bioresearch Inc. Orangebury, New York) : $0.7.10^{-6}$ M; when injected directly into the rat, this AI solution gave a typical blood pressure response of angiotensin II, AI being in vivo immediately converted in AII. This test has been used to estimate the solution's activity. 4) D-aldosterone (Aldocorten® Ciba) : 2.10^{-6} M; 5) glucagon (Glucagon Novo):

1. $8 \cdot 10^{-6}$ M; cyclic AMP (Adenosin-3-5'-monophosphoric acid, cyclic C. F. Boehringer Mannheim): $4 \cdot 6 \cdot 10^{-3}$ M; 7) dibutyryl-acyclic AMP ($N^{6,2'}O$ -Dibutyryl adenosin-3':5' monophosphonic acid, cyclic, C. F. Boehringer Mannheim): $5 \cdot 3 \cdot 10^{-3}$ M; 8) Vasopressin (ADH) (Vasopressin (ADH) (Vasopressin® Sandoz) : 330 mU/ml. 9) Theophylline (Theophyllin-7-sodium acetate Fluka AG) was prepared at a concentration of $10 \cdot 10^{-3}$ M with KR.

2. Determination of Renin Activity of Incubation Media :

Procedures to estimate renin activity in incubation media consisted in incubating 10 ml aliquot of incubation medium at pH 5.5 in presence of 2.5 ml homologous renin substrate (pool of human plasma) and Dowex resin 50W-X2 (NH_4^+), for three hours at $37^\circ C$ (12). The angiotensin produced by the reaction of renin on the renin substrate and adsorbed by the resin is a function of in vitro RR by the kidney slice.

Angiotensin was measured by a rat bio-assay. These procedures are similar to the Boucher's method (13).

In order to ensure that only renin activity released during the experiment was measured, some of the incubation media were directly assayed on the rat, prior to the incubation. The pressor activity possibly thus obtained was subtracted from the total pressor activity measured after determination of renin activity.

In a later experimental phase, instead of determining the pressor activity before the incubation for renin activity the media were dialysed at $4^\circ C$ during 3 days against 10 liters of a solution of K-R. (Dialysis tube : calibre 18, Kalle A.G. Wiesbaden-Biebrich, Germany). This procedure permitted to eliminate all known vasoactive polypeptides and amines such as NE and AII prior to renin activity determination.

a) Renin substrate : A pool of human plasma is obtained from the blood of eight normal male subjects aged 27-46 years on unrestricted diet and with normal physical activity. The blood was drawn in the afternoon and diluted in an ammonium solution EDTA (38 g/L).

b) Units used to express renin liberated in vitro : According to the Boucher's method plasma renin activity (PRA) was expressed in nanogramms of angiotensin produced by one liter of plasma incubated and by one minute of the incubation time. The same unit referred to the weight in grammes of fresh kidney cortex slice incubated : ng/L/min/g was used.

RESULTS

a) Control experiments (tables I and II, fig. 1)

Twelve different kidneys were incubated. RR during incubation period I, representing the basal RR, ranged from 0 to 1586 ng/L/min/g. Basal RR of cortex kidney slices from renal arterial thrombosis (Cases No 1 and 9), malignant hypertension (cases No 2, 8 and 12) and chronic glomerulonephritis with intractable hypertension (case No 11) was elevated, (range : 125 to 1586 ng/L/min/g during incubation period I). On the contrary basal RR was low to undetectable in the other instances (cases No 4, 5, 6, 7 and 10). Human kidney slices incubated in vitro for over 2 hours appeared to release renin at a fairly stable rate; indeed in 9 control experiments mean RR during incubation period II, III and IV expressed in percentage of RR measured during incubation period I, was 83,8 % (II), 73,4 % (III) and 73,1 % (IV). In these control experiments it was impossible to detect any pressor activity of the incubation media when injected to the biological preparation before they had been dialysed. In our experience, human renin does not react with the rat renin substrate used for the detection of pressor activity in the bioassay. This observation is in contrast with an observation reported by Finkelman who demonstrated in vitro angiotensin production by renal glomeruli (14).

b) Effect of NE, NE plus Th, cyclic AMP and dibutyryl cyclic AMP (tables I, II III and IV, fig. 2)

In 13 experiments performed with 8 different kidneys, the adjunction of NE at a concentration of 4.10^{-6} M to the incubation medium during incubation period II, increased the net mean RR by 262,5 % ($P < 0.05$). In 8 of them, this rise ranged between twice to 10 times. Return to basal RR during periods III and IV occurred in all but one case (mean RR during period III : 99,1 %, IV : 87,9 %).

In 7 experiments performed with 2 different kidneys, NE was added to incubation period II at the same concentration (4.10^{-6} M) in conjunction with Th at a concentration of 10.10^{-3} M (table III). Th is a well known inhibitor of cyclic nucleotide phosphodiesterase, that increases, therefore, intracellular cyclic AMP (15). Here again an increase of net RR was observed, fairly of the same magnitude as with NE alone. Mean RR of period II, as percentage change from period I, was 200,6 %. In this small series of 7 experiments it was remarkable that the restitution to basal RR was not as constant as with NE alone. Indeed, in 5 cases among 7, RR remained high after withdrawal of NE and Th (mean percentage of incubation III : 177,3 %, incubation IV : 119,0 %).

In preliminary experiments cyclic AMP was assayed twice without any effect on RR. A possible explanation is that cyclic AMP in respect to dibutyryl cyclic AMP did not get into the target cells, or because of its susceptibility to hydrolysis by phosphodiesterase (15).

Finally in 4 experiments, dibutyryl cyclic AMP (table IV), a mediator of NE analogue to cyclic AMP, (15) was added to the incubation period II, at a concentration of : 5mM. Here again an increase of RR was observed. Mean RR as percentage change from incubation I was 193,7 % ($P < 0.05$).

c) Effect of A II (table I and II, fig. 3)

In 17 experiments the adjunction of A II at a concentration of $0.7 \cdot 10^{-6} \text{M}$ to the incubation medium of period II produced a net decrease of RR of 60 %. In 11 of them RR during period II was less than 50 %, whereas in 15 control experiments only one value was below 50 %. Return to basal release levels (periods III and IV) occurred in all except 2 cases. The reversible inhibition of RR by A II suggests a specific pharmacological effect on renin producing cells and not an unspecific physical noxious action.

d) Effect of A I (table V and fig. 4)

In a small series of 6 experiments conducted simultaneously, both effects of A I and A II were compared. Addition of angiotensin II at a concentration of $0.7 \cdot 10^{-6} \text{M}$ produced the expected inhibition of RR, that is a decrease to half of the basal value, (period II : 49.8 %, $P < 0.01$). In restitution period III an increase of RR was observed in all 6 experiments (period III : 96.3 %). In contrary when A I was added at a concentration of $0.7 \cdot 10^{-6} \text{M}$, the results of period II are dispersed with no value below 50 %, (mean RR during period II : 91.2 %).

e) Effect of G (table VI)

It has been demonstrated with liver cells that G effect is mediated by cyclic AMP, as it is for catecholamines on several organs. In 5 experiments on three different kidneys the addition of G, at a concentration of $1.8 \cdot 10^{-6} \text{M}$ to incubation period II, produced a very strong inhibition of net RR, mean RR during period II was 9.9 %, that is a much more important inhibition than with A II. This inhibition was reversible in all experiments, and highly significant from control experiments performed simultaneously ($p < 0.001$).

f) Effect of ADH (table VII), d-aldosterone (table VIII).

ADH at a concentration of 330 mU/ml during period II had no effect on RR, that remained unchanged, as compared to the controls; d-aldosterone, at a concentration of $2 \cdot 10^{-6} \text{M}$ had also no inhibitory effect. In order to test an possibly retarded effect of d-aldosterone, in 2 of the 4 experiments the addition of the hormone was repeated during period III and IV, with no significant changes on RR in respect of control experiments.

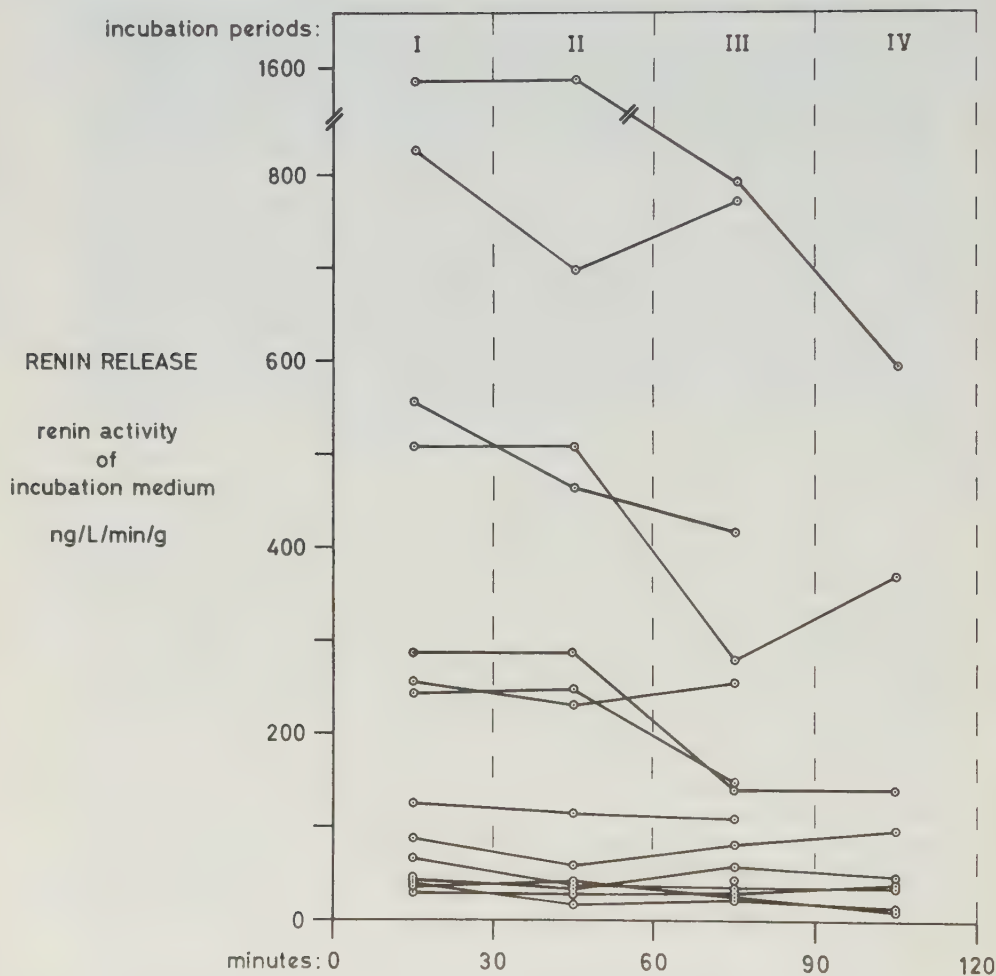
IN VITRO RENIN RELEASE

Fig. 1. Control experiments In vitro renin release expressed in renin activity of the incubation medium by gram of fresh tissue incubated (ng/l/min/g), during four incubation periods of 30 min (I, II, III, and IV).

IN VITRO RENIN RELEASE : EFFECT OF NOREPINEPHRINE

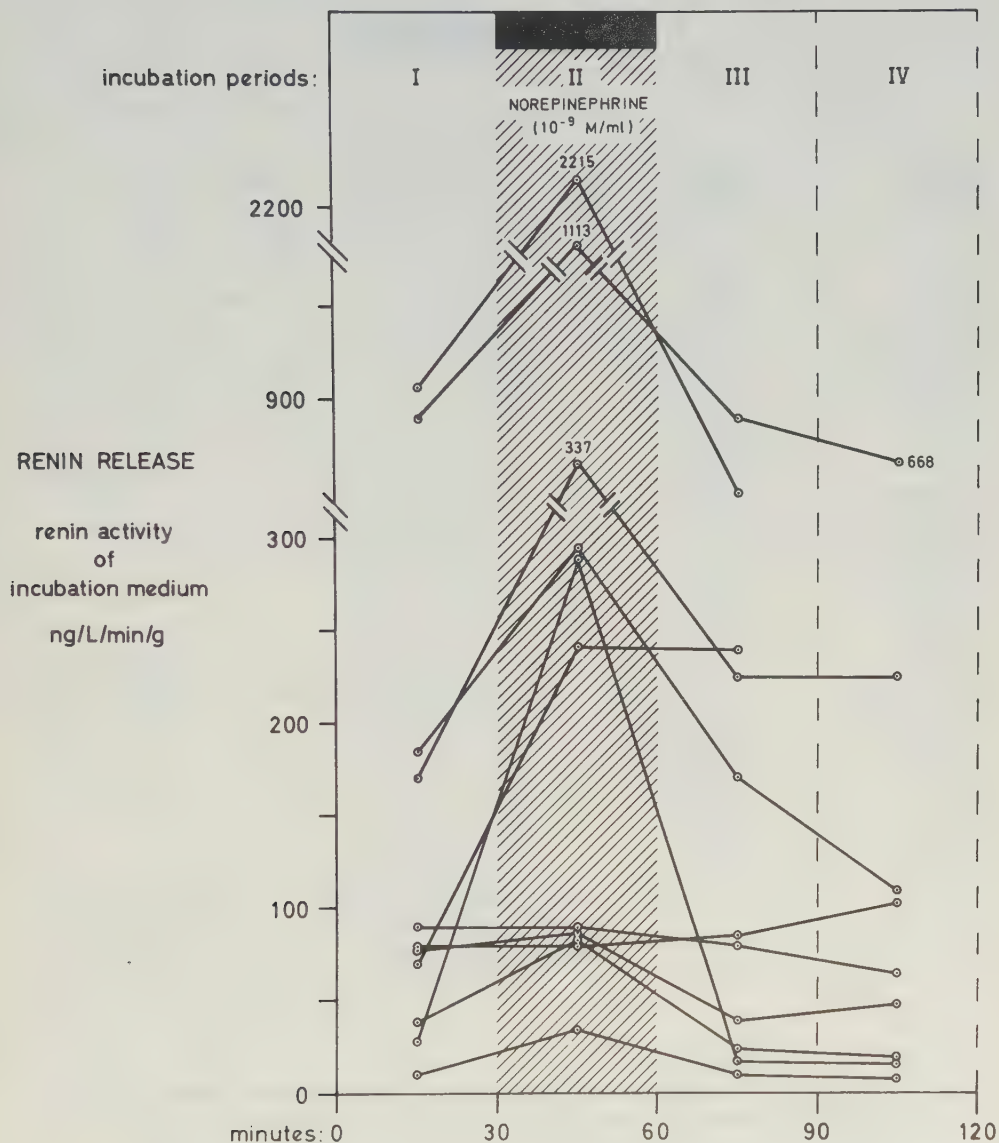


Fig. 2. Effect of norepinephrine (NE) on in vitro renin release expressed in renin activity of the incubation medium by gram of fresh tissue incubated (ng/l/min/g), added during incubation period II.

IN VITRO RENIN RELEASE : EFFECT OF ANGIOTENSIN II

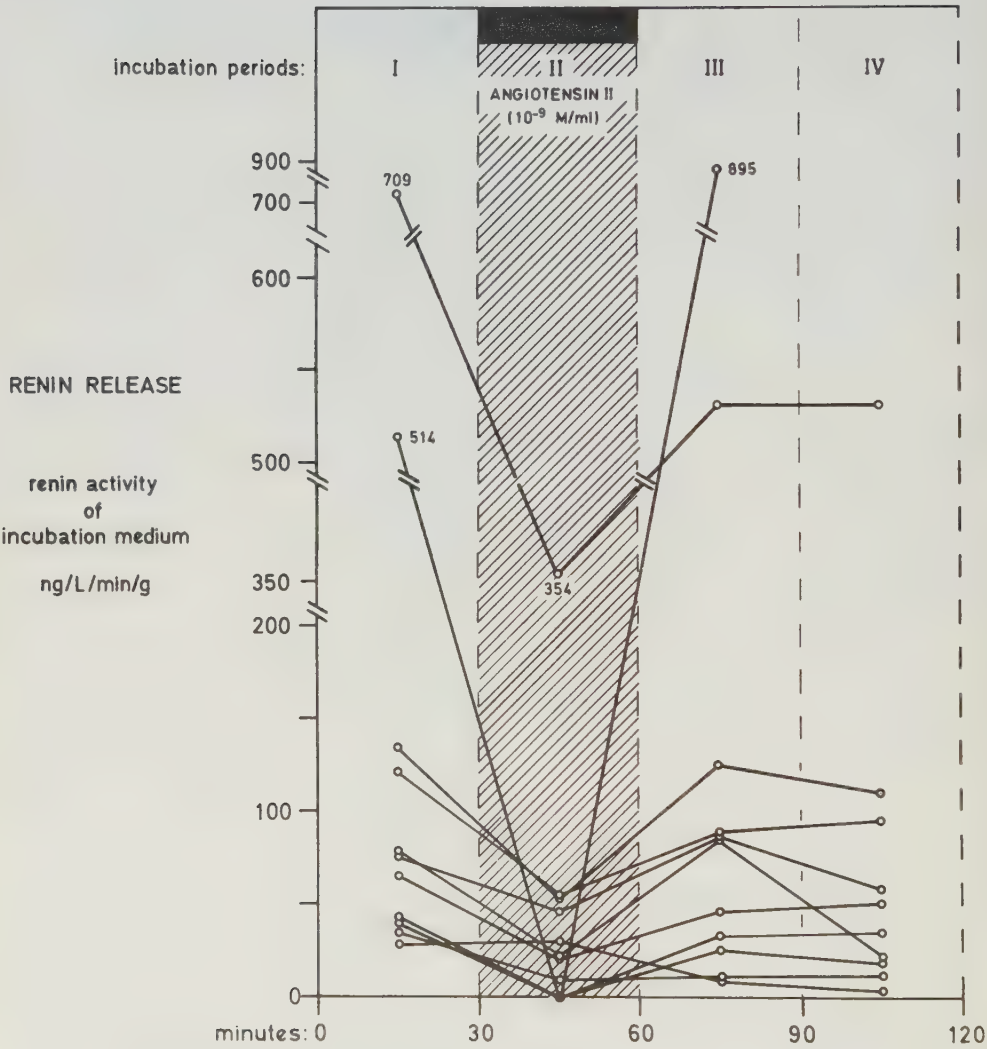


Fig. 3. Effect of angiotensin II (A II) on in vitro renin release expressed in renin activity of the incubation medium by gram of fresh tissue incubated (ng/l/min/g), added during incubation period II.

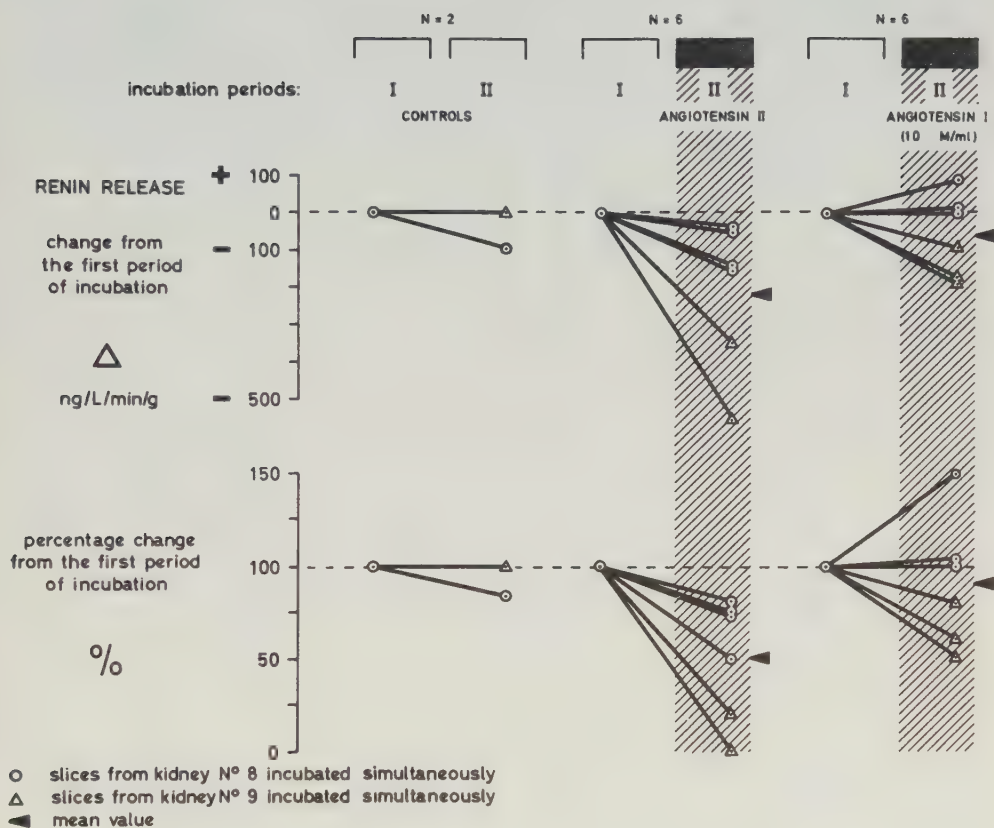


Fig. 4. Comparative effects of angiotensin II (A II) and angiotensin I (A I) on in vitro renin release expressed : 1) in renin activity of the incubation medium by gram of fresh tissue (ng/l/min/g); 2) as percentage change from the first incubation period.

Table I. In vitro renin release : Effect of norepinephrine and angiotensin II. Results expressed in renin activity of the incubation medium by gram of fresh tissue incubated (ng/l/min/g)

Kidney No	Controls				Norepi- nephrine 10 ⁻⁶ M				Angio- tension II 10 ⁻⁶ M			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
1	242	246	147	--	907	2215	850	--	514	0	895	--
	827	698	772	--	--	--	--	--	--	--	--	--
2	125	113	110	--	69	242	241	--	--	--	--	--
	255	231	255	--	--	--	--	--	--	--	--	--
3	41	34	58	48	77	87	40	50	121	54	88	94
	87	58	81	99	90	90	81	65	134	53	124	109
	--	--	--	--	185	296	172	111	75	46	87	58
	--	--	--	--	78	80	86	104	65	22	46	50
4	65	36	35	45	28	289	18	18	42	-5	25	18
	--	--	--	--	--	--	--	--	--	--	--	--
5	29	28	30	40	38	83	25	22	40	-71	33	35
	41	18	23	14	10	35	11	10	34	8	11	11
7	40	43	23	10	63	123	93	93	28	30	9	4
	--	--	--	--	37	82	46	22	76	22	87	22
8	556	463	417	--	--	--	--	--	185	150	293	--
	--	--	--	--	--	--	--	--	309	154	309	--
	--	--	--	--	--	--	--	--	185	139	185	--
	--	--	--	--	--	--	--	--	509	370	462	--
9	509	509	278	370	--	--	--	--	347	0	231	185
	--	--	--	--	--	--	--	--	694	139	431	185
10	45	35	45	--	--	--	--	--	--	--	--	--
11	284	284	142	142	169	337	225	225	--	--	--	--
12	1586	1586	793	595	891	1113	891	668	709	354	532	532
N = 15					N = 13				N = 17			
m =	315.5	292.1	213.9	--	203.2	390.2	213.8	--	239.2	90.6	226.4	--
SD	423.8	415.2	256.8	---	312.9	616.5	301.1	--	233.5	116.5	240.2	--
SEM	109.4	107.2	66.3	--	86.8	171.0	83.5	--	56.6	28.3	58.3	--
p*	--	NS	NS	--	--	NS	NS	--	--	<0.02	NS	--
N = 9					N = 11				N = 12			
m =	298.0	288.4	162.6	151.4	151.5	237.7	153.5	162.2	197.1	60.7	142.0	108.6
SD	509.1	514.2	250.4	200.2	251.4	308.8	253.5	190.2	250.8	100.1	171.2	147.3
SEM	169.7	171.4	83.5	66.7	75.8	93.1	76.4	57.3	72.4	28.9	49.4	42.5
p*	--	NS	NS	NS	--	NS	NS	NS	--	NS	NS	NS

* From t test on paired observations.

NS : non specific

Table II. In vitro renin release : Effect of norepinephrine and angiotensin II.
Results expressed as pourcentage change from the first period of incubation (100 %)

Kidney No	Controls				Norepi- nephrine 10-6 M				Angio- tensin II 10-6 M			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
1	100	102	61	--	100	244	94	--	100	0	174	--
	100	84	93	--	--	--	--	--	--	--	--	--
2	100	90	88	--	100	351	349	--	--	--	--	--
	100	91	100	--	--	--	--	--	--	--	--	--
3	100	83	141	117	100	113	52	65	100	45	73	78
	100	67	93	114	100	100	90	72	100	40	93	81
	--	--	--	--	100	160	93	60	100	61	116	78
	--	--	--	--	100	103	110	133	100	34	71	77
4	100	55	55	69	100	1032	64	64	100	0	60	43
	--	--	--	--	--	--	--	--	--	--	--	--
5	100	97	103	138	100	218	66	58	100	0	82	88
	100	44	56	34	100	350	110	100	100	26	32	32
7	100	108	58	25	100	195	148	148	100	107	32	14
	--	--	--	--	100	222	124	59	100	29	114	30
8	100	83	75	--	--	--	--	--	100	81	158	--
	--	--	--	--	--	--	--	--	100	50	100	--
	--	--	--	--	--	--	--	--	100	73	91	--
9	100	100	55	73	--	--	--	--	100	0	67	53
	--	--	--	--	--	--	--	--	100	20	62	27
10	100	78	100	--	--	--	--	--	--	--	--	--
11	100	100	50	50	100	199	133	133	--	--	--	--
12	100	100	50	38	100	125	100	75	100	50	75	75
N = 15					N = 13				N = 17			
$\bar{m}$ =	100	85.5	78.5	--	100	262.5	117.9	--	100	40.6	88.2	--
SD	--	18.2	26.6	--	--	245.6	74.7	--	--	31.9	37.9	--
SEM	--	4.7	6.9	--	--	68.1	20.7	--	--	7.7	9.2	--
P*	--	NS	NS	--	--	<0.05	NS	--	--	<0.05	NS	--
N = 9					N = 11				N = 12			
$\bar{m}$ =	100	83.8	73.4	73.1	100	256.1	99.1	87.9	100	34.3	73.1	56.3
SD	--	23.0	31.9	41.0	--	267.4	30.1	34.4	--	30.5	26.5	26.0
SEM	--	7.7	10.6	13.7	--	80.6	9.1	10.4	--	8.8	7.6	7.5
P*	--	NS	NS	NS	--	NS	NS	NS	--	<0.001	<0.01	<0.001

* From t test on paired observations

NS : non specific

Table III. In vitro renin release : effect of norepinephrine (NE)
plus theophylline (Th).

Kidney No	Controls				NE (10 ⁻⁶ M) + TH (10 ⁻³ M)			
	I	II	III	IV	I	II	III	IV
A. Results expressed in renin activity of the incubation medium by gram of fresh tissue incubated (ng/1/min/g)								
9	509	509	278	370	187	493	493	493
	--	--	--	--	1184	1776	774	592
	--	--	--	--	259	296	222	111
10	45	35	45	--	48	92	56	--
					51	82	82	--
					33	60	80	--
					36	103	93	--
$\bar{m}$	277.0	272.0	161.5	--	256.9	414.6	257.1	398.7
SD	--	--	--	--	418.2	620.6	274.9	253.9
SEM	--	--	--	--	158.1	234.6	103.9	146.6
P*	--	--	--	--	--	NS	NS	--
B. Results expressed as percentage change from the first period of incubation (100 %)								
9	100	100	55	73	100	264	264	264
	--	--	--	--	100	150	63	50
	--	--	--	--	100	114	86	43
10	100	78	100	--	100	192	116	--
	--	--	--	--	100	160	160	--
	--	--	--	--	100	343	310	--
$\bar{m}$	--	--	--	--	100	200.6	177.3	119.0
SD	--	--	--	--	--	78.0	95.5	125.6
SEM	--	--	--	--	--	29.5	36.1	72.5
P*	--	--	--	--	--	< 0.01	NS	NS

* From t test on paired observations

NS : non specific

Table IV. In vitro renin release : effect of AMPC and dibutylryl AMPC.

Kidney No	Controls				cyclic AMP 4, 6, 10-3 M				dibutylryl cyclic AMP 5, 3, 10-3 M			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
A. Results expressed in renin activity of the incubation medium by gram of fresh tissue incubated (ng/l/min/g)												
4	65	36	35	45	36	35	34	30	--	--	--	--
5	29	28	30	40	56	46	41	35	--	--	--	--
	41	18	23	14	--	--	--	--	--	--	--	--
7	40	43	23	10	--	--	--	--	46	54	23	20
					--	--	--	--	17	37	20	10
					--	--	--	--	40	99	29	12
$\bar{m}$	43.6	31.3	27.6	27.3	46.0	40.5	37.5	32.5	34.3	63.3	24.0	14.0
SD	15.2	10.6	5.9	17.8	--	--	--	--	15.3	32.0	4.6	5.3
SEM	7.6	5.4	2.9	8.9	--	--	--	--	8.8	18.5	2.6	3.1
P*	--	NS	NS	NS	--	--	--	--	--	NS	NS	NS
B. Results expressed as percentage change from the first period of incubation (100 %)												
4	100	55	55	69	100	97	94	83	--	--	--	--
5	100	97	103	138	100	83	73	62	--	--	--	--
	100	44	56	34	--	--	--	--	--	--	--	--
7	100	108	58	25	--	--	--	--	100	122	50	44
					--	--	--	--	100	212	112	57
					--	--	--	--	100	247	75	30
$\bar{m}$	100	76.0	68.0	66.5	100	90.0	83.5	72.5	100	193.7	79.0	43.7
SD	--	31.3	23.4	51.3	--	--	--	--	--	64.5	31.2	13.5
SEM	---	15.6	11.7	25.7	--	--	--	--	--	37.2	18.0	7.8
P*	--	NS	<0.05	NS	--	--	--	--	--	<0.05	NS	<0.001

*From t test on paired observations

NS : non specific

Table V. In vitro renin release : Effect of angiotensin II and I.

Kidney No	Controls				Angio-tensin II 10-6 M				Angio-tensin I 10-6 M			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
A. Results expressed in renin activity of the incubation medium by gram of fresh tissue incubated (ng/l./min/g)												
8	556	463	417	--	185	150	293	--	309	324	240	--
	--	--	--	--	309	154	309	--	370	370	370	--
	--	--	--	--	185	139	185	--	185	278	170	--
	--	--	--	--	509	370	462	--	--	--	--	--
9	509	509	278	370	347	0	231	185	434	347	322	322
	--	--	--	--	694	139	431	185	363	185	247	247
	--	--	--	--	--	--	--	--	457	278	298	222
	--	--	--	--	--	--	--	--	--	--	--	--
N = 6												
m = 371.5 158.7 318.5 -- 353.0 297.0 274.5 --												
SD 198.4 118.9 109.1 -- 97.9 66.1 70.4 --												
SEM 81.0 48.5 44.5 -- 39.9 26.9 28.7 --												
P* < 0.05 NS -- -- NS NS --												
B. Results expressed as percentage change from the first period of incubation (100 %)												
8	100	83	75	--	100	81	158	--	100	105	78	--
	--	--	--	--	100	50	100	--	100	100	100	--
	--	--	--	--	100	75	100	--	100	150	90	--
	--	--	--	--	100	73	91	--	--	--	--	--
9	100	100	55	73	100	0	67	53	100	80	74	74
	--	--	--	--	100	20	62	27	100	51	68	68
	--	--	--	--	--	--	--	--	100	61	65	49
	--	--	--	--	--	--	--	--	--	--	--	--
N = 6												
m = 100 49.8 96.3 -- 100 91.2 79.5 --												
SD -- 33.1 34.3 -- -- 35.7 13.8 --												
SEM -- 13.6 14.0 -- -- 14.6 5.6 --												
P* -- < 0.01 NS -- -- NS < 0.01 --												

* From t test on paired observations
NS : non specific

Table VI. In vitro renin release : effect of glucagon

Kidney No	Controls				gluca- gon I, 8 10-6 M			
	I	II	III	IV	I	II	III	IV
A. Results expressed in renin activity of the incubation medium by gram of fresh tissue incubated (ng/l/min/g)								
3	41	34	58	48	347	116	154	132
	87	58	81	99	43	7	42	40
4	65	36	35	45	23	0	9	11
	--	--	--	--	13	0	13	8
5	29	28	30	40	77	0	69	77
	41	18	23	14	--	--	--	--
$\bar{m}$	52.6	34.8	45.4	49.2	100.6	24.6	57.4	53.6
SD	23.3	14.7	23.8	30.9	139.9	51.2	59.2	51.9
SEM	10.4	6.6	10.7	13.8	62.6	22.9	26.5	23.2
P*	--	NS	NS	NS	--	NS	NS	NS
B. Results expressed as percentage change from the first period of incubation (100 %)								
3	100	83	141	117	100	33	44	38
	100	67	93	114	100	16	98	93
	100	55	55	69	100	0	39	48
4	--	--	--	--	100	0	100	62
5	100	97	103	138	100	0	90	100
	100	44	56	34	--	--	--	--
$\bar{m}$	100	69.2	89.6	94.4	100	9.9	74.2	68.1
SD	--	21.2	35.9	42.1	--	14.9	29.9	27.4
SEM	--	9.5	16.1	18.2	--		13.4	12.2
P*	--	< 0.02 > 0.01	NS	NS	--	< 0.001	NS	< 0.05 > 0.02

*From t test on paired observations.

NS : non specific

Table. VII. In vitro renin release : effect of ADH

Kidney No	Controls				ADH			
					330 mU/ml			
	I	II	III	IV	I	II	III	IV
A. Results expressed in renin activity of the incubation medium by gram of fresh tissue incubated (ng/1/min/g)								
9	509	509	278	370	494	617	185	247
	--				476	423	317	317
					494	453	370	247
					741	741	463	370
$\bar{m}$					551.3	558.5	333.8	295.3
SD					126.8	148.6	116.1	59.8
SEM					63.4	74.3	58.0	29.9
P*					--	NS	0.02	0.01
B. Results expressed as percentage change from the first period of incubation (100 %)								
9	100	100	55	73	100	125	38	50
					100	89	67	67
					100	92	75	50
					100	100	62	50
$\bar{m}$					100	101.5	60.5	54.3
SD					--	16.3	15.9	8.5
SEM					--	8.2	8.0	4.3
P*					--	NS	< 0.01	< 0.001

*From t test on paired observations.

NS : non specific

Table VIII. In vitro renin release : Effect of D-aldosterone

Kidney No	Controls				D-aldosterone 2.10 ⁻⁶ M				D-aldosterone 2.10 ⁻⁶ M			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
A. Results expressed in renin activity of the incubation medium by gram of fresh tissue incubated (ng/1/min/g)												
3	41	34	58	48	62	70	79	--	--	--	--	--
	87	58	81	99	43	72	58	35	--	--	--	--
9	509	509	278	370	--	--	--	--	741	648	373	373
	--	--	--	--	--	--	--	--	266	270	236	278
B. Results expressed as percentage from the first period of incubation (100 %)												
3	100	83	141	117	100	113	127	--	--	--	--	--
	100	67	93	114	100	98	135	81	--	--	--	--
9	100	100	55	73	--	--	--	--	100	87	50	50
	--	--	--	--	--	--	--	--	100	102	89	105

DISCUSSION

In vitro RR by kidney cortex slices offers the opportunity to study the renin released by the juxtaglomerular apparatus, in exclusion of the hemodynamic variations and tubular cation modifications. This approach of the problem of renin regulation has already been tempted by other authors with mammalian kidney, with cortex slices tissue, homogenates or cell cultures (16, 17, 18, 19, 20, 21, 22, 23, 24, 25), but never with human kidney. At the same time the experimental design allows a kind of "kinetic" estimation of RR during four successive incubation periods of 30 minutes of the same kidney slice. So the same slice can be studied in different situations : basal RR during the first period, RR under hormonal effect during period II, and restitution to basal RR, period III and IV, after withdrawal of the agent tested.

Basal RR in control studies

Human kidney cortex slices incubated in vitro for four consecutive periods release renin into the incubation medium at a fairly constant rate. The enzyme released respects the criteria for human renin. The demonstration that RR is dependant of glucose concentration in the medium (20), that it can be inhibited by cyclohexamide (21) and that the RR can be modified reversibly by the addition to the medium of appropriate stimulators or inhibitors, strongly suggests that net RR is been measured rather than non specific leakage.

The in vitro RR of the kidneys which presented in vivo an activation of the juxtaglomerular apparatus, that is renal arterial thrombosis (cases 1, 3, 9), malignant hypertension (cases 2, 8, 12) and chronic pyelonephritis with intractable hypertension (case II), is higher than RR of kidneys with in vivo non stimulated juxtaglomerular apparatus.

In vitro stimulation of RR

In vitro, the adjunction of NE, NE and Th, or dibutyryl cyclic AMP provokes a significant increase of RR. These results strongly suggest a direct action of the pressor amines on the juxtaglomerular cells, and confirm the evidences obtained in vivo (26, 27, 28, 29, 30), that were never absolutely independant of hemodynamics modifications, like a constriction of the afferent arteriole of the glomerulus.

In the experiments with NE alone, RR returns to basal values in almost every case. On the contrary in those where Th is added to NE, the restitution to basal RR is not constant. The explanation could be, that Th, a methylxanthine, inhibitor of the cyclic phosphodiesterase (15), permits an intracellular increase of cyclic AMP, that persists during incubation period III.

In vitro inhibition of RR

A) Angiotensin II

A II, one of the most potent pressor hormone, produces in vivo (31, 32) at subpressor dose a marked inhibition of plasma renin activity. On the contrary

NE, another pressor hormone, produces an increase of plasma renin activity. Few explanations of this phenomenon have been proposed. One of them, fitting with the baroreceptor theory, is based on a specific vasoconstrictor effect of the catecholamines on the afferent arteriole, and an identical effect of A II on the efferent arteriole of the glomerulus. In this indirect mechanism, the constriction of the afferent arteriole by NE, produces a lowering of the pressure at the level of the juxtaglomerular apparatus, increasing secondarily RR. Inversely, the vasoconstriction of the efferent arteriole by A II, without effect on the afferent arteriole, inhibiting secondarily RR.

But in vivo the two hormones produce an identical characteristic distribution of intrarenal blood flow that cannot fit with this selective effect on the arterioles (33). A direct effect of NE and A II on RR is more likely. Already some experiments in vivo are in favour of this (31, 32).

In vitro A II inhibits markedly RR, and this inhibition is reversible, even at the pharmacological concentration. These results confirm those previously reported by de Jong (18) on the rat and are confirmed by Mikelakis on the dog, with the demonstration of a dose response inhibition of A II (34). The very fast inhibition of A II on RR suggests more an inhibition of the mechanism of active renin release, than an inhibition of production.

B) Angiotensin I

In six experiments, the adjunction of A I, the decapeptide precursor of A II, has no effect on RR, compared to six simultaneously experiments in which A II was added. This absence of effect of A I could be a specificity of the receptors of the renin producing cells for A II. Recent observations of Goodfriend and Lin (35) effectuated with labeled A I and A II demonstrated that specific binding of A II was much greater than that of A I in the uterus and kidney. On the contrary A I showed a greater specific binding capacity for adrenal cortex than A II.

C) Glucagon

It has been demonstrated that the mediator of hormonal effect of G is, at least in the liver, the adenyl cyclase system. Our working hypothesis in studying G on human kidney slices was to see if an increase of RR could be observed, as it was with dibutyryl cyclic AMP. In vivo effect of G has not been much studied in respect to its effect on the renin angiotensin system. Preliminary results obtained with a normal subject show no modification of the PRA with a potent infusion of glucagon over four hours (unpublished data). But increase of RR in isolated rat kidney perfused with a solution of G has been demonstrated (36).

On our in vitro model, on the contrary, a marked inhibition of RR is observed in all experiments. Control experiments conducted simultaneously do not present this inhibition. The difference between these two groups is highly significant. Furthermore the inhibition is reversible, restitution to basal value being observed in all cases. There is no explanation to this phenomenon, and the precise role of glucagon on RR remains obscure.

Drugs without effect on in vitro RR

Angiotensin I has already been discussed in the previous sections on inhibition of the RR.

A) Vasopressin (ADH)

In vivo effect of ADH is controversial (4), in some experiments ADH produces an inhibition of RR (9, 10, 37). In vitro ADH has no effect on RR. An explanation could be that there is a lack of ADH receptors on the renin releasing cells, and therefore the inhibition observed in vivo is secondary to others factors.

B) D-aldosterone

Aldosterone, the end product of the renin-angiotensin-aldosterone system is well known to play a role in renal sodium reabsorption (38). In vivo administration of d-aldosterone, in conjunction with a salt diet, decreases PRA to undetectable levels. This effect is not immediate but takes a few days. In spite of a prolonged period of exposition (90 min) of the kidney slices to d-aldosterone, no effect was observed in vitro on RR.

Here again, it suggests that d-aldosterone in vivo effect on PRA level is secondary to others factors, as tubular reabsorption of sodium, that increases extracellular fluid volume.

In conclusion, the above results obtained in vitro, with some drugs known to have an influence in vivo on PRA level suggest that the ones presenting the same effect in vitro as in vivo, act directly on the renin producing cells.

On the contrary, the others drugs that do not have an effect in vitro, are active in vivo on RR through others mechanisms that regulate RR.

SUMMARY

An experimental model has been proposed to study in vitro the renin release (RR) by human kidney cortex slices. This preparation is relatively stable through four successive incubation periods of 30 minutes (incubation I, II, III, IV). Norepinephrine (NE), norepinephrine plus theophylline (Th), and dibutyryl cyclic AMP, added to the second incubation period stimulate RR, whereas angiotensin II (A II) and glucagon (G) in the same conditions inhibit RR. No effect of d-aldosterone, nor of ADH or angiotensin I (A I) was observed. Pharmacological activatory effect of NE and inhibitory effect of A II on in vitro RR is similar to the effect of these two hormones observed in vivo. In vitro effect, consequently independent of hemodynamic or cations variations, suggests a direct effect of NE and A II on the renin producing cells.

RESUME

La libération de rénine (RR) in vitro par des tranches de reins humains a été étudiée dans un modèle expérimental. Cette préparation est suffisamment stable durant quatre périodes d'incubation successives de 30 minutes chacune (incubation I, II, III, IV). En ajoutant à la période d'incubation II de la noradrénaline (NE), de la noradrénaline plus de la théophylline (Th), et de la dibutyryl AMP cyclique, on observe une stimulation de la RR ; au contraire dans des conditions identiques, l'angiotensine II (A II) et le glucagon (G) inhibent la RR. La d-aldostérone, la vasopressine (ADH) et l'angiotensine I (A I) n'ont aucun effet. L'effet pharmacologique activateur de la NE et inhibiteur de l'A II sur la RR in vitro est identique à l'effet produit in vivo par ces 2 hormones. L'effet in vitro, par définition indépendant de variations hémodynamiques ou ioniques, suggère une action directe de la NE et de l'A II sur les cellules produisant la rénine.

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